

WHAT IS CLAIMED IS:

1. A method of measuring the luminescence emitted in a luminescent assay, which comprises employing at least one luminescent tracer compound and a luminescent compound used as an internal reference, which, when exposed to the same excitation wavelength, are capable of emitting at different wavelengths,  $\lambda_2$  and  $\lambda_1$  respectively, either by direct luminescence or by the induction of a luminescent emission, and correcting the measurement of the luminescence emitted by the tracer compound at wavelength  $\lambda_2$  on the basis of the measurement of the luminescence emitted by the reference compound at wavelength  $\lambda_1$ .
2. A method according to claim 1 wherein the luminescent tracer compound and the reference compound are one and the same compound.
3. A method according to claim 1 wherein the luminescent tracer compound and the reference compound are different.
4. A method according to any one of claims 1 to 3 wherein the luminescent tracer compound and/or the reference compound are fluorescent compounds.
5. A method according to any one of claims 1 to 4 wherein the luminescent tracer compound and/or the reference compound are rare earth chelates or cryptates.
6. A method according to any one of claims 1 to 5 wherein the luminescent tracer compound and/or the reference compound have a lifetime of more than one microsecond.
7. A method according to any one of claims 1 to 6 wherein the measurements of the luminescence emitted by the reference compound and by the tracer compound, at wavelengths  $\lambda_1$  and  $\lambda_2$  respectively, are made simultaneously.

TOEFTT "645800T

8. Use of the method according to claim 1 in a homogeneous method of detecting and/or determining an analyte in a medium in which it may be present.

9. Use according to claim 8 in a homogeneous method of detecting and/or determining an analyte in a medium in which it may be present, with the aid of an excess method consisting in:

1) adding, to said medium containing the target analyte, a first reagent made up of at least one receptor for said analyte, coupled with a luminescent donor compound,

2) adding a second reagent made up of one or more other receptors for said analyte, said second reagent being coupled with a luminescent acceptor compound,

3) incubating said medium after the addition of each reagent or after the addition of both reagents,

4) exciting the resulting medium at the excitation wavelength of the luminescent donor compound, and

5) measuring the signal of the luminescent donor compound at a wavelength  $\lambda_1$ , this measurement serving as a reference, and the signal resulting from the energy transfer at a wavelength  $\lambda_2$ .

10. Use according to claim 8 in a homogeneous method of detecting and/or determining an analyte in a medium in which it may be present, with the aid of a competitive method consisting in:

1) adding, to said medium containing the target analyte, a first reagent made up of a receptor for said analyte, coupled with a luminescent donor compound,

2) adding a second reagent made up of the analyte coupled with a luminescent acceptor compound,

3) incubating said medium after the addition of each reagent or after the addition of both reagents,

4) exciting the resulting medium at the

1000549 1111

excitation wavelength of the luminescent donor compound, and

5) measuring the signal of the luminescent donor compound at a wavelength  $\lambda_1$ , this measurement serving as a reference, and the signal resulting from the energy transfer at a different wavelength  $\lambda_2$ .

11. Use according to claim 8 in a homogeneous method of detecting and/or determining an analyte in a medium in which it may be present, with the aid of a competitive method consisting in:

1) adding, to said medium containing the target analyte, a first reagent made up of a receptor for said analyte, said receptor being coupled with a luminescent acceptor compound,

2) adding a second reagent made up of the analyte coupled with a luminescent donor compound,

3) incubating said medium after the addition of each reagent or after the addition of both reagents,

4) exciting the resulting medium at the excitation wavelength of the luminescent donor compound, and

5) measuring the signal of the luminescent donor compound at a wavelength  $\lambda_1$ , this measurement serving as a reference, and the signal resulting from the energy transfer at a different wavelength  $\lambda_2$ .

12. Use according to claim 8 in a homogeneous method of detecting and/or determining an analyte in a medium in which it may be present, with the aid of a method consisting in:

1) adding to said medium a first reagent made up of a receptor for said analyte,

2) adding a second reagent selected from the analyte or at least one of its receptors, one of the two reagents being coupled with a luminescent tracer compound and the other reagent containing a heavy atom or moieties containing a heavy atom, as well as a

TOEFT 645800T

luminescent compound serving as an internal reference,

3) incubating the resulting medium either after the addition of each reagent or after the addition of both reagents,

5 4) exciting the resulting medium, and

5) measuring on the one hand the signal emitted by the luminescent tracer compound, said signal being modulated by the heavy atom effect at a wavelength  $\lambda_2$ , and on the other hand the signal emitted by the reference compound at a wavelength  $\lambda_1$ .

10 13. Use according to any one of claims 9 to 12 wherein the measurements of the luminescence emitted by the reference compound and by the tracer compound, at wavelengths  $\lambda_1$  and  $\lambda_2$  respectively, are made simultaneously.

15 14. Use according to any one of claims 9 to 11 wherein at least one of the receptors for the analyte is bound to a solid support.

20 15. Device for carrying out the method according to claim 1, which comprises an exciting light source, means for collecting the light beam emitted following said excitation, and means for measuring the luminescence at two different wavelengths.

25 16. Device according to claim 15, which further comprises means for splitting the beam emitted following excitation.

30 17. Device according to one of claims 15 or 16, which also comprises a method of correcting the measurement made at wavelength  $\lambda_2$ , built in the apparatus, consisting in fixing a counting rate on the channel measuring the luminescent emission of the reference compound at wavelength  $\lambda_1$ , and then, when this rate is reached, triggering the end of the measurement on the channel measuring the luminescent  
35 emission at wavelength  $\lambda_2$ .

1000549-1130  
TETTT 645800T

Adcl  
B1